Aquaculture 283 (2008) 141–147

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Processing soybean meal for biodiesel production; effect of a new processing method on growth performance of rainbow trout, *Oncorhynchus mykiss*☆

Frederic T. Barrows [a,*], T. Gibson Gaylord [a], Wendy M. Sealey [b], Michael J. Haas [c], Robert L. Stroup [d]

[a] U.S. Department of Agriculture, Agricultural Research Service, Hagerman Fish Culture Experiment Station, 3059-F National Fish Hatchery Road, Hagerman, ID, 83332, USA
[b] University of Idaho, Hagerman Fish Culture Experiment Station, 3059-F National Fish Hatchery Road, Hagerman, ID, 83332, USA
[c] U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane., Wyndmoor, PA, 19038, USA
[d] R.L. Stroup Co. LTD., 1835 Fox Run, Troy, OH, USA

ARTICLE INFO

Article history:
Received 20 November 2007
Received in revised form 28 June 2008
Accepted 29 June 2008

Keywords:
Soybean meal processing
Biodiesel
Trout feed
Alternate protein source
In situ transesterification

ABSTRACT

A new method of soybean meal processing has been developed, which may simplify the process of biodiesel production. This method, '*in situ* transesterification', eliminates hexane extraction to remove the oil, combining the extraction and transesterification steps so as to synthesize biodiesel via a single treatment conducted directly on a lipid-bearing solid material. If the resulting meal is comparable in nutritional value to commercially available hexane-extracted soybean meal (SE-SBM) the new process could become widely used in the bio-fuel industry. Two levels (17.5 and 35%) of each of three types of soybean meal were fed to triplicate lots of 30 (initial wt 22 g) rainbow trout for 9 weeks in flow-through 15 °C spring water. The three types of soybean meal included SE-SBM, an experimentally produced hexane-extracted SE-SBM (ESE-SBM), and a meal produced using *in situ* transesterification (IS-SBM) and each was fed at two levels for a total of 6 diets. Growth of fish fed the diets was good, averaging over 600% gain. There was no effect of source of soybean meal on weight gain of trout. The fish fed the meal processed by the new method, IS-SBM, gained as much weight as fish fed either of the two control meals, at each inclusion level. Fish fed the diets containing IS-SBM, however, did have higher feed intake (2.51% bw/d) compared to fish fed the ESE-SBM or SE-SBM, 2.38 and 2.46% bw/d, respectively. Since growth was equal, feed conversion ratios were higher for fish fed the IS-SBM diets. Protein and energy retention values were lower for fish fed the IS-SBM diets. There was no effect of soybean source on carcass composition. Apparent digestibility for protein was lower for the IS-SBM (85.9%) than for the ESE-SBM (89.3%). Feeding IS-SBM did not decrease weight gain in this study, but due to the increased feed intake and FCR, long term feeding trials should be conducted to further evaluate the meal.

Published by Elsevier B.V.

1. Introduction

As the aquaculture industry searches for sustainable replacements for fish meal, the energy industry also searches for sustainable replacements for fossil fuels. Both industries are evaluating oilseed crops, but with minimal forethought their uses could be complementary rather than competitive. For example, biodiesel which consists of the simple alkyl esters of fatty acids can be made effectively from soybean oil, but current methods of production are costly. While a replacement for fish oil is needed and soybean oil can be used in aquaculture feeds, affordable high quality soy protein is currently of greater interest.

Soybean meal has been a staple protein source for the animal feed industries but its value has been limited for rainbow trout for a number of reasons. The most prominent factor limiting soybean meal inclusion in trout or salmon feeds has been the occurrence of enteritis (Refstie et al., 2000). The oligosaccharide content of soybean meal, primarily stachyose and raffinose, has been identified as a possible causative agent of soybean enteritis (van den Ingh et al., 1991, 1996; Bureau et al., 1998). Lending support for this theory is the fact that soy protein concentrate contains only ~3% oligosaccharides compared to up to 15% in some soybean meal (Russett, 2002) and soy protein concentrate can be used at higher dietary inclusion levels than soybean meal without reducing growth (Gatlin et al., 2007). A new method for processing soybean meal may affect the chemical composition of the meal and thus affect either the presence of enteritis, or the level at which enteritis occurs in trout fed the new meal.

An additional limitation to the utilization of soybean meal in rainbow trout diets has been that the amino acid balance of soybean meal is slightly low in methionine (NRC, 1993). This limitation can generally be overcome by dietary supplementation with commercially

☆ Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

* Corresponding author.
E-mail address: Rick.Barrows@ars.usda.gov (F.T. Barrows).

0044-8486/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.aquaculture.2008.06.041

Table 1
Composition of reference diet for determining apparent digestibility coefficients

Ingredient	g kg^{-1} (dry weight basis)
Menhaden fish meal, Special Select™[a]	550.0
Wheat flour[b]	344.9
Menhaden Fish oil[b]	80.0
Vitamin C[c]	3.0
Choline Cl 50%[b]	5.0
Vitamin premix[b]	6.0
Trace Mineral[b]	1.0
Yttrium oxide[d]	0.1
Chromic oxide[d]	10.0
Analyzed composition	**g kg^{-1} (dry weight basis)**
Crude protein	456.9
Phosphorus	17.3
Energy (kJ/g)	22.04

[a] Omega Protein Corp., Hammond, Louisiana, USA.
[b] Nelson & Sons Inc., Murray, UT, USA.
[c] Vitamin C as Rovimix® Stay-C® 35, DSM Nutritional Products, Basel, Switzerland.
[d] Sigma-Aldrich Company, St. Louis, Missouri, USA.

available sources of methionine or by utilizing complimentary protein sources. Supplementation thus can improve the protein value of soybean meal and remove some of the potential growth reduction when soy protein concentrates are utilized as the primary protein ingredient (Medale et al., 1998). These factors combined with limitations on the use of fish meal and terrestrial animal by-product meals have further increased the utilization and thereby demand for soy protein meal in feeds for carnivorous fish.

The biodiesel industry has the potential to utilize a vast amount of soybean oil, if production methods can be cost effective. Estimated biodiesel production in 2007 is 400 million gallons (change to metric), and the industry is experiencing rapid growth (National Biodiesel Board, pers. comm.). New methods are being developed that might support cost-effective production and result in large quantities of soybean meal produced by a method different from that traditionally used (Haas et al., 2004, 2007; Haas and Scott, 2007). While the new processing technique effectively produces high quality biodiesel (Haas and Scott, 2007) it may alter the composition and nutritional value of the soybean meal in either a positive or negative way. Therefore, the current trial was performed to assess changes in composition of soybean meal from an experimental *in situ* transesterification process for oil removal compared to solvent extraction methodology, assess effects of processing technique on apparent nutrient digestibility for rainbow trout, and to measure production performance of rainbow trout fed increasing levels of the *in situ* esterified soybean meal.

2. Materials and methods

2.1. Soybean meal processing

Two soybean meals were experimentally produced. The preparation of *in situ* extracted soybean meal (ISE-SBM) was as previously described (Haas and Scott, 2007). Flakes were dried in a convection oven to less than 1% moisture, determined gravimetrically. In a typical reaction, 2.25 kg of dried flakes was then mixed by gentle rolling at room temperature in a sealed container with 5.75 L of 0.1 N dry NaOH in anhydrous methanol. After 5.5 h the agitation was stopped and the liquid removed by filtration. The flakes were then washed twice by suspension for 5 min in 3.2 L of anhydrous methanol, and recovered by filtration. The bulk of the residual alcohol in the flakes was removed by air drying in shallow pans. Multiple runs were conducted to accumulate 30 kg of ISE-SBM. The meal was then subjected to regimes of heat and steam. Determination of lipid content by extraction and high performance liquid chromatography (Haas et al., 2007), indicated an average residual meal oil content of 1.1% (range: 0.2 to 2.2%).

Experimental hexane-extracted soybean meal (ESE-SBM) differed from the *in situ* extracted soybean meal (ISE-SBM) in that the flaked soybean meal used in preparation of the *in situ* extracted soybean meal was extracted with hexane as opposed to sodium hydroxide and methanol to remove the lipid. Final lipid levels, determined by exhaustive extraction with hexane in a Soxhlet extractor, were less than 1.0% in ESE-SBM. As with ISE-SBM, the ESE-SBM was subsequently treated with heat/moisture regimes and residual levels of trypsin inhibitor activity determined.

2.2. Soybean meal digestibility

To determine the digestible nutrients in the two sources of experimentally produced soybean meals, the methods of Cho et al. (1982) and Bureau et al. (1999) were used to estimate apparent digestibility coefficients (ADCs). Yttrium oxide served as the inert maker. A complete reference diet meeting or exceeding all known nutritional requirements for rainbow trout (NRC, 1993) (Table 1) was blended with the test ingredients (ISE-SBM or ESE-SBM) in a 70:30 ratio (dry weight basis) to form test diets. All diets were produced by cold-extrusion (Advanced Hydrolyzing Systems, Inc., Astoria, OR, USA) with a 3-mm die and dried to <10% moisture.

Rainbow trout, *Oncorhynchus mykiss*, Housecreek strain, were obtained from the College of Southern Idaho (Twin Falls, ID) with 50, 250-g fish per 140-L fiberglass tank. Water temperature was maintained at 14.5 °C throughout the feeding trial using flow-through spring water. Lighting was maintained on a 14:10-h diurnal cycle. Each diet was fed to three tanks of fish. Each diet was randomly assigned to a tank of fish and fed to apparent satiation twice daily for 7 days prior to fecal collection. Fecal samples were obtained in one collection by manual stripping 16–18 h post-feeding. Manual stripping of fish was accomplished by netting and anesthetizing all fish in the tank, followed by gently drying and then applying pressure to the lower abdominal region to express fecal matter into a plastic weighing pan. Care was taken to exclude urinary excretions from the collection. Fecal samples for a given tank were dried overnight at 50 °C and stored at −20 °C until chemical analyses were performed.

Apparent digestibility coefficients of each nutrient in the test diet and ingredients were calculated according to the following equations (Kleiber, 1961; Forster, 1999):

$$\mathrm{ADCN_{diet}} = 100{-}100\{\%\mathrm{Yt\,in\,diet} \times \%\mathrm{nutrient\,in\,feces}\}/\{\%\mathrm{Yt\,in\,feces} \times \%\mathrm{nutrient\,in\,diet}\}$$

$$\mathrm{ADCN_{ingredient}} = \{(a+b)\mathrm{ADCN_t} - (a)ADCN_\mathrm{r}\}b^{-1}$$

where,

$\mathrm{ADCN_{ingredient}}$	apparent digestibility coefficient of the nutrient in the test ingredient
$\mathrm{ADCN_t}$	apparent digestibility coefficients of the nutrient in the test diets
$\mathrm{ADCN_r}$	apparent digestibility coefficients of the nutrient in the reference diet
a	$(1-p)\times$nutrient content of the reference diet
b	$p\times$nutrient content of the test ingredient
p	proportion of test ingredient in the test diet

2.3. Soybean meal utilization

A three by two factorial design was used with soybean meal source and level of soybean meal in the diet as the main effects. Three sources of soybean meal included commercially produced, hexane-extracted soybean meal (SE-SBM), *in situ* extracted soybean meal (ISE-SBM) and experimental hexane-extracted soybean meal (ESE-SBM). The same

Table 2
Ingredient composition (g/kg) of experimental diets

	Soy 17.5	Soy 35
Ingredient		
Fish meal[a]	182.2	182.2
Blood meal[b]	50.0	50.0
Soybean meal[c]	175.0	350.0
Corn gluten meal	133.3	38.4
Wheat gluten	35.7	10.3
Wheat flour	270.7	214.7
Fish oil	120.5	122.0
Di-calcium phosphate	16.2	13.9
Methionine	2.4	4.5
Vitamin premix[d]	5.0	5.0
Choline Cl	6.0	6.0
Ascorbic acid	2.0	2.0
Trace mineral premix[e]	1.0	1.0
Nutrient composition		
Crude protein, g/kg[f]	420.0	416.4
Lipid, g/kg[f]	109.9	105.8
Ash, g/kg[f]	60.9	68.7
Energy, kJ/g	22.3	21.8

[a] Peruvian anchovy, 70% protein.
[b] Spray dried, 70% protein, International Ingredients, Fairfield, NJ.
[c] Soybean meal was either commercially solvent extracted soybean meal (SE-SBM), experimentally solvent extracted soybean meal (ESE-SBM) or *in situ* esterified soybean meal (ISE-SBM).
[d] Contributed per kilogram of diet: vitamin A (as retinol palmitate), 10,000 IU; vitamin D_3, 720 IU; vitamin E (as DL-%-tocopheryl-acetate), 530 IU; niacin, 330 mg; calcium pantothenate, 160 mg; riboflavin, 80 mg; thiamin mononitrate, 50 mg; pyridoxine hydrochloride, 45 mg; menadione sodium bisulfate, 25 mg; folacin, 13 mg; biotin, 1 mg; vitamin B_{12}, 30 mg.
[e] Contributed in mg/kg of diet: zinc, 37; manganese, 10; iodine, 5; copper, 3.
[f] Wet weight basis.

source of soybeans was used to produce both the ISE-SBM and ESE-SBM.

Each of the three meal sources was included in the diet at either 17.5 or 35% of the diet (Table 2), and chosen to be above and below the generally regarded threshold of soybean meal inclusion (20%) that causes intestinal enteritis. The diets were formulated to contain 18% fish meal and other ingredients common in commercial trout feeds, to meet or exceed known nutrient requirements for trout (NRC, 1993) and to be *iso*-nitrogenous and *iso*-lipidic, with 40% crude protein and 12% crude fat.

Prior to mixing the diets, all ingredients were ground using an air-swept pulverizer (Jacobsen 18H, Minneapolis, MN). Dry ingredients were mixed in a horizontal mixer and a portion (~1/3) of the added oil was mixed into the dry ingredients along with the lecithin. The mash was then extruded through a 3.0-mm die of a Buhler twin-screw cooking extruder (DNDL-44, Buhler AG, Uzwil, Switzerland). Barrel temperature averaged 130 °C in sections 2–6, die pressure was ~360 psi and the feed had a barrel residence time of approximately 18 s. The diets were dried in a pulse bed drier (Buhler AG, Uzwil, Switzerland) with air discharge temperature remaining below 104 °C to a final moisture content less than 8%. After the diets were dried, they were top-coated with the remaining oil (8%) using a vacuum coater (A.J. Mixing International, Ontario, CA) and stored at room temperature (~18–23 °C).

Groups of 35 rainbow trout (House Creek strain, College of Southern Idaho), with an average initial weight of 22 g, were randomly placed in each of 18, 150-L fiberglass tanks. Each tank was supplied with 8 L/min of untreated, constant temperature (14.5 °C), gravity-fed spring water at the Hagerman Fish Culture Experiment Station, University of Idaho. There were three tanks of fish per diet and fish were fed three times per day, 6 days per week to apparent satiation for a period of 64 days. A 14-h photoperiod, controlled by timers and fluorescent lights was provided. The experimental protocol was approved by the University of Idaho's Animal Care and Use Committee.

2.4. Chemical analyses

Feed and fecal samples were dried, and analyzed using AOAC (1995) methods for proximate composition, with the exception of protein and lipid. Crude protein (N×6.25) was determined in ingredients, diets and feces by the Dumas method (AOAC, 1995) on a Leco TruSpec N nitrogen determinator (LECO Corporation, St. Joseph, MI, USA). Crude fat was analyzed using a Soxhlet extraction apparatus (Soxtec System HT, Foss Tecator AB, Hoganas, Sweden) with methylene chloride as the extracting solvent, and ash by incineration at 550 °C in a muffle furnace. Total energy was determined by isoperibol bomb calorimetry (Parr1281, Parr Instrument Company Inc., Moline, IL, USA). Yttrium analyses were conducted at the University of Idaho Analytical Sciences Laboratory, Moscow, ID, using an Optima 3200 radial inductively-coupled plasma atomic emission spectrometer (Perkin-Elmer Corp., Norwalk, CT). Amino acids were analyzed by AAA Laboratories (Mercer Island, WA) on a Beckman 6300 AA analyzer (Beckman Instruments, Inc., Fullerton, CA).

The three sources of soybean meal were analyzed for the following constituents by the Archer Daniels Midland Company, Specialty Feeds Division (Decatur, IL): trypsin inhibitor activity (ISO 14902) (AOCS, 1983); stachyose, raffinose, and oligosaccharides concentrations were determined using HPLC and a calcium column; group A saponins were separated and quantified using methods described by Shiraiwa et al. (1991a); group B saponins were separated and quantified using methods described by Shiraiwa et al. (1991b), Hu et al. (2002), and Dalluge et al. (2003).

2.5. Performance indices

The concentrations of moisture, crude protein (N×6.25), energy and phosphorus in the feed and fish at the beginning and end of the study were determined as described above. The amount of protein and total energy fed during the study was used to calculate apparent protein and energy retention during the 64-day study. Indices are expressed on a per-fish basis for each dietary treatment group. Performance indices were calculated using the following formulae:

$$\text{Feed conversion ratio(FCR)} \\ = \text{feed intake(dry weight)/body weight gain(wet weight)}$$

$$\text{Hepatosomatic index(HSI)} = \text{liver mass(g)*100/fish mass(g)}$$

$$\text{Apparent protein retention(PRE\%)} \\ = (\text{protein gain in fish(g)/protein intake in feed(g)}) \times 100$$

$$\text{Apparent energy retention(ERE\%)} \\ = (\text{energy gain in fish(cal)/energy intake in feed(cal)}) \times 100.$$

Feed intake expressed as a percent of body weight per day was calculated as a percentage of the average of the initial and final weights per fish from each tank.

2.6. Histology

Fish fed each of the experimental diets were sampled at the end of the trial for histological analyses. Five fish from each of the replicate tanks were euthanized and samples of kidney, liver, and pyloric and rectal intestines were preserved in Davidson's solution for 48 h. Tissues were then transferred to 65% alcohol until processed by standard histological procedures (Sheehan and Hrapchek, 1983).

2.7. Statistical analyses

Fish performance, nutrient retention, nutrient digestibility, and carcass composition data were analyzed using the general linear models procedure of the Statistical Analysis System (SAS, 1988).

Table 3
Chemical composition of soybean meals

	SE-SBM	ESE-SBM	IS-SBM
Anti-nutrients			
Glycinine, mg/kg	25,000	10,000	25,000
Beta con-glycinine, mg/kg	30,000	9,000	30,000
Lectins, mg/kg	110.0	50.0	180.0
Dry solids, g/kg	925.15	902.94	907.31
Stachyose, g/kg	57.51	61.03	56.52
Raffinose, g/kg	17.11	11.62	16.23
Oligosaccharides, g/kg	74.62	72.66	72.75
TIA, mg/g	4.7	1.80	5.30
Saponin, type B Ba, g/kg	0.245	0.115	0.165
Saponin, type Bb′ (type I), g/kg	0.464	0.082	0.184
Saponin, type Bb (type I), g/kg	1.937	0.721	1.343
Saponin, type B Bc (type II, III), g/kg	0.925	0.335	0.633
Saponin, type B Bc′ (type II, III), g/kg	0.093	0.040	0.075
Type B saponins, g/kg	4.093	1.519	2.92
Type DDMP saponins, g/kg	2.200	0.247	1.564
Total type B and DDMP saponins, g/kg	6.293	1.766	4.484
Nutrients			
Protein, %[a]	48.3	49.8	49.9
Energy, cal/g	NA	4961	4947
Moisture, %	7.0	11.5	11.3
Ash, %[a]	5.8	6.0	6.7
Arginine[b]	7.58	9.08	9.04
Histidine[b]	2.51	2.66	2.69
Isoleucine[b]	4.41	4.79	2.66
Leucine[b]	7.49	8.08	7.90
Lysine[b]	6.35	6.11	6.36
Methionine[b]	1.40	1.28	1.12
Phenylalanine[b]	5.03	5.42	5.33
Threonine[b]	3.90	4.26	4.24
Tyrosine[b]	3.63	4.05	3.89
Valine[b]	5.26	5.08	4.93
Leucine[b]	7.49	8.08	7.90
Lysine[b]	6.35	6.11	6.36
Methionine[b]	1.40	1.28	1.12
Phenylalanine[b]	5.03	5.42	5.33
Threonine[b]	3.90	4.26	4.24
Tyrosine[b]	3.63	4.05	3.89
Valine[b]	5.26	5.08	4.93

[a] Wet weight basis.
[b] Expressed as a percentage of the protein.

Differences in treatments means were separated using the Tukey's multiple range test. Any value expressed as a percentage was arcsine transformed prior to analysis (Sokal and Rohlf, 1981). Data were also analyzed as a 3 by 2 factorial treatment design to evaluate main effects rather than just treatment means. When a significant interaction of the main effects was detected, treatment means were compared using Tukey's multiple range test within dietary inclusion levels.

3. Results

3.1. Chemical composition of the soybean meals

Concentration of major nutrients in the soybean meals was similar, but protein content of ESE-SBM (49.8%) and ISE-SBM (49.9%) was slightly higher than of the commercial source (SE-SBM, 48.3%) on a wet weight basis and much higher on a dry matter basis (Table 3). The experimentally produced meals had higher moisture content (avg. 11.4%) but did not mold before incorporation into the experimental diets and were then thoroughly dried. The protein content of the SE-SBM diets was 42.4% compared to 41.4% for the ESE-SBM diets and 41.7% for the ISE-SBM diets. Amino acid concentrations of the three soybean meals were also similar (Table 3). Trypsin inhibitor activity, glycinine, beta con-glycinine, lectins, various carbohydrates and saponins were determined for each of the meals (Table 1). The concentrations of each of these substances, which are commonly referred to as anti-nutrients, were found in the ISE-SBM to be approximately equivalent to the levels found in either the SE-SBM or the ESE-SBM (Table 3).

3.2. Apparent digestibility and availability coefficients

The apparent digestibility coefficients (ADC) for organic matter, dry matter, energy and phosphorus were all similar for the ESE-SBM and ISE-SBM (Table 4). The ADC for protein was significantly higher for the ESE-SBM (87.3%) than for the IS-SBM (85.9%).

Significant differences were detected for apparent amino acid availability coefficients (AAC) between the soybean meals processed by the different methods (Table 4). The AAC for four essential amino acids, arginine, isoleucine, leucine, and threonine were higher for the ESE-SBM than for ISE-SBM. The opposite trend was observed for non-essential amino acids. The AAC for aspartic acid, serine and proline was higher in the ISE-SBM than in ESE-SBM (Table 4).

3.3. Fish performance and nutrient retention

There were effects of dietary soybean meal source and level on growth, FCR and feed intake (Table 5). The level of soybean meal in the diet significantly affected growth; trout fed the diets with 35% soybean meal had greater growth (126.5 g) than the fish fed the 17.5% soybean meal diets (119.1 g). There was a trend for an effect of source of soybean meal on weight gain ($P=0.09$). Trout fed diets containing SE-SBM had an average gain of 124.2 g compared to 123.0 g fish fed diets with ESE-SBM, or 121.3 g for trout fed diets with ISE-SBM (Table 6).

FCR for fish fed all diets ranged from 0.86 to 0.98 g feed/g gain (Table 5). There was an effect of both level and source of soybean meal on FCR. The fish fed the diets containing 17.5% soybean meal had significantly better FCR than fish fed the 35% soybean meal diets. The fish fed the IS-SBM diets had the highest FCR of 0.93 compared to 0.90 for fish fed the SE-SBM or 0.87 for fish fed the ESE-SBM diets (Table 6). A significant interaction of level and source on FCR also was observed. At 17.5% SBM, the FCR for fish fed the IS diets was not different from fish fed either SE or ESE-SBM. When diets contained 35% soybean meal, fish fed the ISE diets had significantly higher FCR than fish fed either SE or ESE (Table 5).

Source and level of soybean meal in the diet also had an affect on feed intake (Table 6). Trout fed diets containing ISE-SBM consumed more feed (2.51% bw/d) than fish fed the diet with SE-SBM (2.46% bw/d) or the diets with ESE-SBM (2.38% bw/d). Increasing the inclusion level of soybean meal from 17.5 to 35% resulted in an increase in feed intake, from 2.39 to 2.51% bw/d. There was no significant interaction of these two effects.

Neither source nor inclusion level of soybean meal had an effect on HSI or fillet yield (Tables 5 and 6). There were effects of soybean meal source and level on both PRE and ERE. Fish fed diets with ESE-SBM had PRE values of 43.9%, compared to 41.8 and 40.7% for fish fed SE-SBM and ISE-SBM, respectively. Increasing dietary inclusion level from 17.5 to 35% resulted in an increase in PRE from 41.3 to 43.0% (Table 6). This same increase in inclusion level, however, resulted in a decrease of ERE from 42.3% for fish fed diets with 17.5 to 40.9% for the fish fed diets with 35% SBM. Source of soybean meal did not affect ERE, but significant interactions of the main effects were observed. When diets containing 17.5% SBM were fed, there was no difference in ERE among the three sources. When diets containing 35% SBM were fed, fish fed the ISE-SBM diets had significantly lower ERE (38.3%) compared to 42.5 and 41.8% for fish fed the SE-SBM and ESE-SBM diets, respectively (Table 5).

3.4. Body composition

Soybean meal source did not affect body composition, but level of soybean meal in the diet had an effect on body protein, lipid and energy content. The body protein content increased significantly when the level of soybean meal increased from 17.5 to 35%. Body lipid

Table 4
The effect of source of soybean meal on the apparent digestibility coefficients and amino acid availability coefficients

ADC or AAC (%)	ESE-SBM	+SD	IS-SBM	+SD	R-square	$P>F$
Organic matter	69.7	0.8	67.1	1.6	0.58	0.13
Dry matter	65.4	1.0	61.9	1.8	0.65	0.10
Protein	87.3	0.6	85.9	0.1	0.88	0.02
Energy	75.1	0.6	72.2	1.2	0.75	0.06
Phosphorus	48.3	6.8	51.9	9.2	0.05	0.72
Arginine	96.7	0.3	95.5	0.4	0.78	0.05
Histidine	93.0	1.0	91.4	0.9	0.53	0.16
Isoleucine	94.6	0.5	91.7	1.0	0.82	0.03
Leucine	94.9	0.5	92.1	0.8	0.85	0.03
Lysine	92.9	1.2	93.6	1.2	0.13	0.55
Methionine	93.4	1.0	92.7	2.2	0.04	0.73
Phenylalanine	93.4	1.4	91.4	1.5	0.44	0.22
Threonine	91.6	0.6	88.0	1.2	0.83	0.03
Tyrosine	92.3	1.5	90.0	3.1	0.23	0.41
Valine	93.1	0.1	90.4	1.6	0.65	0.10
Tryptophan	91.8	1.5	93.1	1.6	0.22	0.43
Glycine	83.5	0.8	87.2	0.5	0.92	0.01
Aspartic acid	87.6	0.8	89.8	1.0	0.71	0.07
Serine	90.5	1.1	93.4	0.8	0.77	0.05
Alanine	91.2	0.5	91.3	0.7	0.02	0.81
Glutamic acid	92.8	0.6	94.1	0.5	0.68	0.08
Proline	87.2	1.3	91.5	0.6	0.86	0.02

and energy content decreased with increasing level of soybean meal in the diet (Table 5). Soybean meal source and level had no effect on body ash or mineral (Ca, P, Mg, K, Na, S) content (Table 5).

3.5. Histology

There was no difference in incidence or severity of the soybean meal-induced enteritis among fish fed the different sources of soybean meal within inclusion level. Although soybean enteritis was observed in fish irregardless of dietary treatment, the severity was much greater for trout fed diets containing 35% soybean meal. Large colonies of gram positive bacteria were observed in the intestine of fish fed diets containing 35% soybean meal. Some bacteria was observed in the intestines of trout fed the 17.5% diets, but incidence and numbers were less than that found in fish fed the 35% soybean meal diets.

4. Discussion

Sustainability is becoming increasingly important in both the aquaculture and energy industries. The search for alternative protein sources to fish meal, and the search for alternatives to fossil fuels have both lead in the same direction focusing on oilseeds. A new method of soybean meal processing has been developed, which could provide benefit to both industries and may simplify the process of biodiesel production. If the resulting meal is comparable in nutritional value to commercially available hexane-extracted soybean meal (SE-SBM) the new process could become widely used in the bio-fuel industry. The results of this study indicate that the new processing methods did not significantly alter the proximate composition or the anti-nutrient content of the resultant soybean meals.

Further, because the value of a feed ingredient cannot be judged by chemical composition alone, *in vivo* comparison of the meals in growth and digestibility trials were conducted and results indicate no effect of type of soybean meal on weight gain of trout. Weight gain of trout fed the experimental diets was good during the 9-week study ranging from 520 to 570% increase over initial body weight. In different studies at this laboratory using fish of equivalent size, similar growth has been observed. In 9 weeks trout fed a diet containing 15% soybean meal gained 450% of initial weight (Gaylord et al., 2006). Gains of 450% were observed when trout were fed diets containing approximately 50% soybean meal for 10 weeks (Barrows et al., 2007). In a longer study, trout gained over 600% of initial weight after 12 weeks (Stone et al., 2005).

The TIA (define) levels (Table 1) observed in the ESE-SBM (1.8) were well below the maximum suggested levels for salmon by Olli et al. (1994). Levels of TIA comparable to these were not found to affect growth or nutrient digestibility in rainbow trout (Olli and Krogdahl, 1994). The TIA level of IS-SBM of 5.3% may have had an affect on growth as suggested by significantly lower ADC for protein (Table 4). An ADC for protein of 87.3% was observed for trout fed the diets with ESE-SBM compared to 85.9% for fish fed the IS-SBM diets. Romarheim et al. (2006) fed diets containing 246 g/kg of soybean meal with a TIA level of 0.6, however, and also observed a decrease in weight gain of Atlantic salmon. Other factors in addition to TIA may be affecting growth performance.

The trout fed the soybean meal produced by *in situ* transesterification had higher feed conversion ratio and feed intake. Compared to fish fed the ESE-SBM, FCR was about 7% higher for fish fed the diet with IS-SBM. This is a slight difference, but would be significant when extrapolated over a production cycle. Increased supplementation of the IS-SBM with amino acids may have improved performance of fish fed the meal produced by *in situ* transesterification.

While growth, in general, in this experiment was high, and an effect of soybean meal inclusion level was expected, trout fed the diets containing 35% soybean meal grew unexpectedly faster than trout fed diets with 17.5% soybean meal. An inclusion level of 20% soybean meal is generally considered to be the threshold for rainbow trout, above which a reduction in growth will be observed. The opposite was observed in this study. Analysis of the essential amino acid content of the diets may explain the observed results. The fish meal content of the experimental diets was kept low at 18.2%, to reflect diets formulated during fish meal shortages. Arginine, threonine, lysine and methionine increased as dietary soybean meal increased from 17.5 to 35% of diet. Arginine content of the diets with 17.5% soybean meal was 6.97% of protein, as compared to 8.02% for the 35% SBM diets. Because of the high level of plant products in the diets, both diets contained more arginine than reported as required for juvenile rainbow trout (NRC, 1993). Reported values are 3.3% (Kaushik, 1979), 3.6% (Walton et al., 1986), 4.0% (Kim et al., 1983), 4.7% (Cho et al., 1989), and 5.9% (Ketola, 1983). Threonine levels of 4.5 and 4.8% of the diet were found in the 17.5 and 35% SBM diets, respectively, and are again considerably higher than 3.0% that has been reported to be required by chum salmon (Akiyama et al., 1985). It is thus unlikely that variations in arginine and threonine levels between the diets with 17.5 and 35% soybean meal were the cause for differences in growth observed for fish fed the diets.

Lysine and methionine levels showed a similar pattern to arginine and threonine levels. Diets with 17.5% SBM averaged 5.2% lysine and 2.5% methionine compared to 6.1% lysine and 3.0% methionine for diets with 35% SBM. While lysine levels in diets with 17.5% SBM were much higher than the reported requirement of 3.7% by Kim and Kayes (1982) and 4.2% by Walton et al. (1984), and lower than 6.1% by Ketola (1983). Methionine requirements less than found in diets with 17.5% SBM have been reported to be 2.2% (Walton et al., 1984), 1.4% (Kim et al., 1992), and 1.5% (Cowey et al., 1992). The methionine content of 2.5% in the diet with 17.5% SBM should have been more than adequate. However, Rumsey et al. (1983) and Kim et al. (1992) reported requirements of 3.0 and 2.9%, respectively. The methionine levels of the 17.5% SBM diets were the same as predicted by the formulation, but the 35% SBM diets were approximately 0.20% of the diet higher than expected. Since all three diets containing 35% SBM had similar methionine levels, the error was probably not a mixing error but more likely due to an inaccurate value for one or more ingredients during formulation. The rapid growth of trout in this study may have also lead to detection of growth differences among fish fed diets with small differences in amino acid content.

All of the reported requirements listed above were determined by feeding either purified or chemically defined diets. The diets in the present study included practical ingredients and synthetic methionine, and with a rapidly growing strain of trout, the accepted methionine requirement (NRC, 1993) may not be adequate. In addition to growth,

Table 5
Effect of diet on growth performance, nutrient retention and body composition of rainbow trout[1]

Soy meal level, %	17.5%			35%			SEM	*P*
Soy meal source	SE	ESE	IS	SE	ESE	IS		
Growth performance								
Gain, g/f	121.7^{ab}	120.7^{ab}	115.0^{b}	126.7^{a}	125.3^{a}	127.7^{a}	1.3	0.01
FCR	$0.92^{b,m}$	$0.86^{c,n}$	$0.87^{bc,mn}$	$0.88^{bc,y}$	$0.88^{bc,y}$	$0.98^{a,x}$	0.1	0.01
Feed intake[2]	2.51^{bc}	2.35^{cd}	2.31^{d}	2.42^{cd}	2.41^{cd}	2.70^{a}	0.8	0.01
HSI, %	1.83	1.57	1.59	1.64	1.48	1.73	0.3	0.15
Fillet yield, %	52.0	50.8	52.0	52.8	52.8	53.0	0.7	0.10
Nutrient retention								
PRE, %[3]	$39.9^{b,m}$	$42.4^{ab,m}$	$41.7^{ab,m}$	$43.7^{ab,x}$	$45.5^{a,x}$	$39.7^{b,y}$	0.6	0.01
ERE, %[4]	$41.3^{ab,m}$	$43.2^{a,m}$	$42.5^{ab,m}$	$42.5^{ab,x}$	$41.8^{ab,x}$	$38.3^{b,y}$	0.3	0.01
Body composition								
Protein[5]	15.2^{ab}	15.4^{ab}	15.3^{ab}	16.6^{a}	16.2^{a}	16.1^{a}	0.2	0.05
Lipid[5]	11.8^{ab}	12.0^{a}	11.5^{ab}	10.7^{ab}	9.0^{b}	11.2^{ab}	0.5	0.02
Ash[5]	2.1	2.2	2.2	2.2	2.0	2.2	0.1	0.63
Moisture	70.7	70.8	71.1	70.9	71.3	71.3	0.3	0.75
Energy, kJ/g	27.5	27.4	27.1	26.8	26.7	26.6	45.3	0.08
Ca (µg/g)	14,166	16,000	16,666	15,666	16,000	10,500	1752	0.77
P (µg/g)	15,166	15,833	16,666	16,500	16,166	15,333	813	0.81
Mg (µg/g)	973	1013	985	1011	1063	985	28	0.44
K (µg/g)	12,000	12,333	12,333	12,833	12,666	12,500	397	0.77
Na (µg/g)	3183	2955	3517	3133	3250	3267	262	0.86
S (µg/g)	6250	6566	6550	6650	6583	6366	272	0.91

[1]Means (n=3, pooled standard error of the mean) in the same row with the same superscript are not significantly different (P>0.05), and superscripts of a, b, and c represent a comparison of all 6 diets. When a significant interaction is detected, superscripts of m, n, and o represent a comparison of sources within the 17% level, and superscripts of x, y, and z represent a comparison of sources within the 35% level.
[2]% body weight per day.
[3]Protein retention efficiency.
[4]Energy retention efficiency.
[5]% Wet weight basis.

other performance data also support a possible marginal amino acid deficiency causing the reduced growth for trout fed the 17.5% soybean meal diets. Feed intake was significantly greater for fish fed the 35% SBM diets as compared to trout fed the 17.5% SBM diets. Medale et al. (1998) observed a decrease in both feed intake and growth of rainbow trout due to a methionine deficiency in a diet containing high levels of soy protein concentrate. Body protein content was significantly higher (16.3%) and body lipid content was lower (10.8%) for trout fed the 35% SBM diets compared to 15.3% body protein and 11.8% body fat for trout fed the 17.5% diets. Cheng et al. (2003) found similar results when feeding reduced fish meal diets to rainbow trout. Feeding diets with increased plant proteins and decreased methionine levels resulted in a decrease in body protein concentration. Crystalline methionine was added to the diets in the present study, and this form of the amino acid may not be used as efficiently as methionine from intact protein as was observed for channel catfish with crystalline lysine (Zarate and Lovell, 1997). The data from the current study suggest that with practical ingredients, and only 18% fish meal and 0.24% synthetic methionine, a total dietary level of 1.0% methionine and 0.50% cystine may be inadequate for maximum growth.

Despite the differences in feed intake and body composition, growth for fish fed the 17.5% diets was good, suggesting just a marginal deficiency. The statistical strength of the factorial analyses is demonstrated with these data, since the effect of inclusion level was not apparent using the multiple range test, but was significant following factorial analysis.

Histological results from the current study are consistent with those reported by Baeverfjord and Krogdahl (1996) and Refstie et al. (2000) with regards to intestinal enteritis. Severity of the enteritis increased with increasing levels of dietary soybean meal, regardless of the source or type of soybean meal processing. *In situ* transesterification did not seem to alleviate or worsen this cellular response. The presence of Gram positive bacteria in the trout intestine in the present study is also

Table 6
The effect of soybean meal processing method and diet inclusion rate on feed consumption and growth efficiency of rainbow trout[a]

	Source of meal			Level, %		Probability>*F* value				*R*- square	
	SE	ESE	IS	17.5	35	Model	Source	Level	S*L[b]		CV
Growth performance											
Gain, g/f	124.2	123.0	121.3	119.1	126.5	0.01	0.09	0.01	0.07	0.77	2.32
FCR	0.90	0.87	0.93	0.88	0.91	0.01	0.01	0.01	0.01	0.87	2.14
Feed intake, %bw/d	2.46	2.38	2.51	2.39	2.51	0.01	0.03	0.01	0.12	0.90	2.10
HSI, %	1.73	1.52	1.66	1.66	1.61	0.10	0.06	0.48	0.15	0.49	8.46
Fillet yield, %	52.4	51.8	52.4	51.6	52.8	0.79	0.84	0.21	0.88	0.16	3.95
Nutrient retention											
PRE, %[c]	41.8	43.9	40.7	41.3	43.0	0.01	0.01	0.04	0.01	0.73	3.62
ERE, %[d]	41.9	42.5	40.4	42.3	40.9	0.05	0.15	0.05	0.05	0.55	4.16
Body composition											
Protein %	15.9	15.8	15.7	15.3	16.3	0.05	0.84	0.01	0.61	0.52	3.84
Lipid, %	11.3	11.2	11.4	11.8	10.8	0.02	0.85	0.01	0.16	0.63	4.46
Energy, cal/g	27.2	27.2	26.8	27.3	26.7	0.04	0.36	0.01	0.83	0.58	1.38

[a] Means (n=3, pooled standard error of the mean) in the same column with the same superscript are not significantly different (P>0.05).
[b] Source by level interaction.
[c] Protein retention efficiency.
[d] Energy retention efficiency.

consistent with feeding soybean meal to salmon. Bakke-McKellep et al. (2007) reported changes in the bacterial population due to inclusion of soybean meal in the diet. There were higher total numbers of bacteria and more diversity in bacterial populations with fish fed the higher level of soybean meal. In the present study, or in the study of Bakke-McKellep et al. (2007), a direct involvement of the bacteria in causing the enteritis could not be determined, but should be evaluated further.

5. Conclusions

This study indicates that soybean meal produced through *in situ* transesterification can be a suitable substitute for traditional hexane-extracted soybean meal, but attention must be given to amino acid balance.

Acknowledgements

This research was funded in as part of the USDA/Agricultural Research Service, Trout-Grains Project 5366-21310-003-00D. We thank Mike Casten and Lorrie VanTassel for fish care, Karen Scott for conducting the *in situ* transesterification on soybeans, and Lucas Porter for assistance in feed processing. We also thank John Less and ADM Corporation for soybean meal analyses.

References

Akiyama, T., Arai, S., Murai, T., Nose, T., 1985. Threonine, histidine and lysine requirements of chum salmon fry. Bull. Jpn. Soc. Sci. Fish. 51, 635–639.
AOAC, 1995. Official Methods of Analysis. Association of Official Analytical Chemists, Inc., Arlington, Virginia, USA.
AOCS (American Oil Chemists' Society), 1983. In: Walker, R.C. (Ed.), Official methods and tentative practices of the American Oil Chemists. AOCS, Champaign, IL. 840 pp.
Baeverfjord, G., Krogdahl, A., 1996. Development and regression of soybean meal induced enteritis in Atlantic salmon distal intestine. A comparison with intestines of fasted fish. J. Fish Dis. 19, 375–387.
Bakke-McKellep, A.M., Penn, M.H., Salas, P.M., Reftsie, S., Sperstad, S., Landsverk, T., Ringo, E., Krogdahl, A., 2007. Effects of dietary soyabean meal, inulin and oxytetracycline on intestinal microbiota and epithelial stress, apoptosis and proliferation in the teleost Atlantic salmon (*Salmo salar* L.). Brit. J. Nutr. 97, 699–713.
Barrows, F.T., Stone, D.A.J., Hardy, R.W., 2007. The effects of extrusion conditions on the nutritional value of soybean meal for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 265, 244–252.
Bureau, D.P., Harris, A.M., Cho, C.Y., 1998. The effects of purified alcohol extracts from soy products on feed intake and growth of chinook salmon and rainbow trout. Aquaculture 161, 27–43.
Bureau, D.P., Harris, A.M., Cho, C.Y., 1999. Apparent digestibility of rendered animal protein ingredients for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 180, 345–358.
Cheng, Z.J., Hardy, R.W., Blair, M., 2003. Effects of supplementing methionine hydroxyl analogue in soybean meal and distillers dried grain-based diets on the performance and nutrient retention of rainbow trout (*Oncorhynchus mykiss*). Aquac. Res. 34, 1303–1310.
Cho, C.Y., Slinger, S.J., Bayley, H.S., 1982. Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. Comp. Biochem. Physiol. 73B, 25–41.
Cho, C.Y., Kaushik, S.J., Woodward, B., 1989. Dietary arginine requirement of rainbow trout. FASEB 3, 2459 (abstract).
Cowey, C.B., Cho, C.Y., Sivak, J.G., Weerheim, J.A., Stuart, D.D., 1992. Methionine intake in rainbow trout (*Oncorhynchus mykiss*), relationship to cataract formation and metabolism of methionine. J. Nutr. 122, 1154–1163.
Dalluge, J.P., Eliason, E., Frazer, S., 2003. Simultaneous identification of soyasaponins and isoflavones and quantification of soyasaponin Bb in soy products, using liquid chromatography/electrospray ionization-mass spectrometry. J. Agric. Food Chem. 51, 3520–3524.
Forster, I., 1999. A note on the method of calculating digestibility coefficients of nutrients provided by single ingredients to feeds of aquatic animals. Aquac. Nutr. 5, 143–145.
Gatlin III, D.M., Barrows, F.T., Bellis, D., Brown, P., Campen, J., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G., Krogdahl, ., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., Souza, E., Stone, D., Wilson, R., Wurtele, E., 2007. Expanding the utilization of sustainable plant products in aquafeeds — a review. Aquacult. Res. 38, 551–579.
Gaylord, T.G., Teague, A.M., Barrows, F.T., 2006. Taurine supplementation of all-plant protein diets for rainbow trout (*Oncorhynchus mykiss*). J. World Aquacult. Soc. 37, 509–517.
Haas, M.J., Scott, K.M., 2007. Moisture removal substantially improves the efficiency of *in situ* biodiesel production from soybeans. J. Am. Oil Chem. Soc. 84, 197–204.
Haas, M.J., Scott, K.M., Warmer, W.N., Foglia, T.A., 2004. *In situ* alkaline transesterification: an effective method for the production of fatty acid esters from vegetable oils. J. AOCS 81, 83–89.
Haas, M.J., Scott, K.M., Foglia, T.A., Marmer, W.N., 2007. The general applicability of *in situ* transesterification for the production of fatty acid esters from a variety of feedstocks. J. Am. Oil Chem. Soc. 84, 963–970.
Hu, J., Lee, S., Hendrich, S., Murphy, P.A., 2002. Quantification of the group B soyasaponins by high performance liquid chromatography. J. Agric. Food Chem. 50, 2587–2594.
Kaushik, S., 1979. Application of a biochemical method for the estimation of amino acid needs in fish. Quantitative arginine requirements for rainbow trout in different salinities. In: Tiews, K., Halver, J.E. (Eds.), Finfish Nutrition and Fishfeed Technology. Heenemann GmbH, Berlin, pp. 197–207.
Ketola, H.G., 1983. Requirement for dietary lysine and arginine by fry of rainbow trout. J. Anim. Sci. 56, 101–107.
Kim, K.I., Kayes, T.B., 1982. Test diet development and lysine requirements of rainbow trout. FAESEB 41, 716 (abstract).
Kim, K.I., Kayes, T.B., Amundson, C.H., 1983. Protein and arginine requirements of rainbow trout. FAESEB 42, 2198 (abstract).
Kim, K.I., Kayes, T.B., Amundson, C.H., 1992. Requirements for sulfur containing amino acids and utilization of D-methionine by rainbow trout (*Oncorhynchus mykiss*). Aquaculture 101, 95–103.
Kleiber, M., 1961. The fire of life: an introduction to animal energetics. John Wiley and Sons, Inc., New York, NY, USA.
Medale, F., Boujard, T., Vallee, F., Blanc, D., Mambrini, M., Roem, A., Kaushik, S.J., 1998. Voluntary feed intake, nitrogen and phosphorus losses in rainbow trout (*Oncorhynchus mykiss*) fed increasing dietary levels of soy protein concentrate. Aquat. Living Resour. 11, 239–246.
National Research Council (NRC), 1993. Nutrient Requirements of Fish. National Academy Press, Washington, DC. 114 pp.
Olli, J.J., Krogdahl, A., 1994. Nutritive value of four soybean products as protein sources in diets for rainbow trout (*Oncorhynchus mykiss*, Walbaum) reared in freshwater. Acta Agric. Scand. 44, 185–192.
Olli, J.J., Hjelmeland, K., Krogdahl, A., 1994. Soybean trypsin inhibitors in diets for Atlantic salmon (*Salmo salar*, L.): effect on nutrient digestibility and trypsin in pyloric caeca homogenate and intestinal content. Comp. Biochem. Physiol. 109A, 923–928.
Refstie, S., Korsoen, O.J., Storebakken, T., Baeverfjord, G., Lein, I., Roem, A.J., 2000. Differing nutritional responses to dietary soybean meal in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). Aquaculture 190, 49–63.
Romarheim, O.D., Skrede, A., Gao, Y., Krogdahl, A., Denstadli, V., Lileeng, E., Storebaken, T., 2006. Comparison of white flakes and toasted soybean meal partly replacing fish meal as protein source in extruded feed for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 256, 354–364.
Rumsey, G.L., Page, J.W., Scott, M.L., 1983. Methionine and cystine requirements of rainbow trout. Prog. Fish-Cult. 45, 139–143.
Russett, C., 2002. Soy protein concentrates for animal feeds. Animal nutrition research notes. The Solae. SPC-T-47, Fort Wayne, IN. 12 pp.
SAS Institute, 1988. SAS Procedures Guide, 6.03 edn. SAS Institute, Cary, NC. 441 pp.
Sheehan, D., Hrapchek, B., 1983. Theory and Practice of Histotechnology, 2nd Ed. Battlelle Press, Columbus, OH.
Shiraiwa, M., Kudo, S., Shimoyamada, M., Harada, K., Okubo, K., 1991a. Composition and structure of “Group A saponin” in soybean seed. Ag. Biol. Chem. 55, 315–322.
Shiraiwa, M., Harada, K., Okubo, K., 1991b. Composition and structure of “Group B saponin” in soybean seed. Ag. Biol. Chem. 55, 911–917.
Sokal, R.R., Rohlf, F.J., 1981. Biometry, 2nd ed. W.H. Freeman and Company, New York, NY. 857 pp.
Stone, D.A., Hardy, R.W., Barrows, R.W., Cheng, Z.J., 2005. Effects of extrusion on nutritional value of diets containing corn gluten meal and corn distiller's dried grain for rainbow trout, *Oncorhynchus mykiss*. J. Appl. Aquac. 17, 1–20.
van den Ingh, T.S.G.A.M., Krogdahl, Å., Olli, J.J., Hendriks, H.G.C.J.M., Koninkx, J.G.J.F., 1991. Effects of soybean-containing diets on the proximal and distal intestine in Atlantic salmon: a morphological study. Aquaculture 94, 297–305.
van den Ingh, T.S.G.A.M., Olli, J.J., Krogdahl, Å., 1996. Alcohol soluble components in soybeans cause morphological changes in the distal intestine of the Atlantic salmon, *Salmo salar*. J. Fish Dis. 19, 47–53.
Walton, M.J., Cowey, C.B., Adron, J.W., 1984. The effect of dietary lysine levels on growth and metabolism of rainbow trout (*Salmo gairdneri*). Brit. J. Nutr. 52, 115–122.
Walton, M.J., Cowey, C.B., Coloso, R.M., Adron, J.W., 1986. Dietary requirements of rainbow trout for tryptophan, lysine and arginine determined by growth and biochemical measurements. Fish Physiol. Biochem. 2, 161–169.
Zarate, D.D., Lovell, R.T., 1997. Free lysine (L-lysine HCL) is utilized for growth less efficiently than protein-bound lysine (soybean meal) in practical diets by young channel catfish (*Ictalurus punctatus*). Aquaculture 159, 87–100.